Effectiveness of electrochemically activated water as an irrigant in an infected tooth model

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Abstract

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Aim To test the effectiveness of electrochemically activated aqueous solutions in the debridement of *Enterococcus faecalis* biofilms in root canals of extracted teeth.

Methodology Extracted, human, single-rooted teeth (198) assembled into 11 sets ($n = 18$) with matching anatomical characteristics were randomly assigned to eight experimental groups. After decoronation, the root canals were prepared to a standard size. *Enterococcus faecalis* biofilms were grown in the root canals of autoclaved, individually mounted teeth over 48 h. Electrolysed saline collected as anolyte at the anode and catholyte at the cathode were the test agents. The four ultrasonication and four without ultrasonication irrigant groups included: neutral anolyte (NA) (pH 6.5), acidic anolyte (AA) (pH 3.0), catholyte (C) (pH 11.5) and C alternated with neutral anolyte (C/NA). Phosphate-buffered saline (PBS) with and without ultrasonication formed negative and NaOCl (3%) positive control groups. After irrigation, root canal samples were serially diluted, cultured and enumerated. The data were analysed as ratios of residual colony-forming units (CFUs) in PBS versus the test irrigants and using multivariate regression.

Results The NA and NA (ultrasonicated, U), C/NA and AA (U) groups had significantly ($\alpha = 0.05$) less and C (U) and C/NA (U) significantly ($\alpha = 0.05$) more bacteria (CFUs mL^{-1}) compared with their respective PBS controls. Ultrasonicated C/NA had significantly ($\alpha = 0.05$) higher CFU counts than the nonultrasonicated solution. Other comparisons between ultrasonic and nonultrasonic groups were not significant. Of the nonultrasonicated groups, C/NA and NA were most effective, whilst of the ultrasonicated groups, AA and NA were most effective. None of these was as effective as 3% NaOCl.

Conclusions All but two groups (AA and C) were significantly different from their PBS controls. There was a significant difference between the C/NA groups with and without ultrasonication but not between other combinations. NA (U) and AA (U) were the most effective test solutions but NaOCl (3%) gave by far the highest bacterial kills.

Keywords: antibacterial, electrochemically activated water, root canal.

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Introduction

Root canal treatment consists of mechanical preparation to achieve a regularly tapered canal access that should facilitate delivery of an antibacterial irrigant to the entire root canal system. Whilst sodium hypochlorite is effective in this task, it is also associated with tooth weakening when used in high concentrations, e.g. 5% (Grigoratos *et al.* 2001, Sim *et al.* 2001) and can be toxic to the periapical tissues (Cvek *et al.* 1976, Becking 1991, Hales *et al.* 2001). There is a need for an irrigant with equivalent antibacterial properties that is milder in its action on vital tissue but whilst ably

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debriding the dentine surface does not weaken its structure in the manner of sodium hypochlorite (O'Driscoll *et al.* 2002). Such an irrigant has reputedly been found and is known as either 'Electrochemically activated water' (Marais 2000, Solovyeva & Dummer 2000) or 'oxidative potential water' (Hata *et al.* 1996, 2001, Serper *et al.* 2001). The solutions are generated by electrolysing saline solution, a process no different to that used in the commercial production of sodium hypochlorite (Frais *et al.* 2001). The difference however is that the solution accumulating at the anode is harvested as the anolyte and that at the cathode as the catholyte. These solutions display properties that are dependent upon the strength of the initial saline solution, the applied potential difference and the rate of generation. The technology that allows harvesting of the respective solutions resides in the design of the anode and the cathode and originates either in Russia (electrochemically activated water) or Japan (oxidative potential water) (Marais 2000). Although the solutions are named differently, the principles of manufacture are probably the same.

The endodontic literature on the use of this technology is sparse but shows early promise. The solutions from both technologies have been tested for their ability to debride root canals (Marais 2000, Solovyeva & Dummer 2000, Hata *et al.* 2001), remove smear layer (Hata *et al.* 1996, Serper *et al.* 2001), kill bacteria (Horiba *et al.* 1999, Marais & Brozel 1999, Marais & Williams 2001, Prince *et al.* 2002) and bacterial spores (Loshon *et al.* 2001), with favourable results whilst showing biocompatibility with vital systems (Ichikawa *et al.* 1999, Petrushanko & Lobyshev 2001, Serper *et al.* 2001).

Anolyte and catholyte solutions generated from one such technology (Radical Waters Halfway House 1685, S. Africa) have shown promise as antibacterial agents against laboratory grown single species biofilm models (Ghori *et al.* 2002). Such solutions have been recommended as suitable for removing biofilms in dental unit water lines (Marais & Brozel 1999) and has even been marketed for this purpose (Walker *et al.* 2003).

Bacteria grown as biofilms maybe up to 1000 times more resistant to killing than their broth-grown counterparts (Wilson 1996) and are regarded as a more relevant test model (Spratt *et al.* 2001). *Enterococcus faecalis* has been implicated in apical periodontitis (Allard *et al.* 1987) as well as root canal treatment failure (Molander *et al.* 1998, Sundqvist *et al.* 1998). It is thought to be amongst the most resistant bacteria associated with root canal infection. A test model consisting of biofilms of *E. faecalis* grown in root canals of extracted teeth was therefore developed to test solutions of electrochemically activated water.

Ultrasonic activation of sodium hypochlorite is known to enhance its debridement efficacy (Cameron 1987) and the same principle could potentially apply to electrochemically activated aqueous solutions because of their chemical similarity. It was therefore decided to evaluate the effect of ultrasonication on various solutions of electrochemically activated water.

This study was designed to answer specific questions about the antimicrobial efficacy of electrochemically activated solutions. These were: (i) Are the experimental solutions better than a control (phosphate-buffered saline, PBS)? (2) Are the experimental solutions activated by ultrasonication better than those not ultrasonicated? (3) Which is the best experimental solution (both with and without ultrasonication)?

Materials and methods

Selection, preparation and allocation of teeth

Extracted, human, single-rooted teeth ($n = 198$) consisting of incisors, canines and premolars, stored in formal-saline (4%) were used to assemble 11 groups ($n = 18$) with matching anatomical characteristics. Length and radiographically estimated canal 'volume' were used to create loosely matching sets; these characteristics were recorded for later analysis. The teeth were decoronated and the root canals prepared using ProFiles.06 taper instruments (Dentsply Maillefer, Ballaigues, Switzerland) starting with tip size 40 and stepping down until tip size 30 reached the canal terminus using water irrigation. Canal patency was established initially by passing a size 08 K file to the apical foramen. If two canals were present then both were prepared and teeth with canals wider than .06 taper were replaced with those that were not. The root ends (2 mm of the tip) were sealed with a dentine bonding agent (Kerr Optibond Solo, Peterborough, UK) and Kerr light cured composite (Kerr Hawe, Peterborough, UK) to close off any apical deltas.

The eleven groups of 18 teeth were randomly allocated to eight experimental groups, four without ultrasonication and four with; the four irrigants were: neutral anolyte (NA) (pH 6.5), acidic anolyte (AA) (pH 3.0), catholyte (C) (pH 11.5) and C alternated with NA. PBS (ultrasonicated and nonultrasonicated groups) acted as negative controls and NaOCl (3%) acted as a positive control.

Preparation of infected tooth model

The teeth were individually mounted in lids of Bijou bottles by cutting holes to create a custom fit for each root. They were secured with 'Trim' acrylic (Bosworth Trim; Bosworth Company, Skokie, IL, USA) and dentine-bonding resin (Kerr Optibond Solo) and allowed to dry for 10 min. The entire assembly was wrapped in tin foil and autoclaved at 121 °C for 15 min. The Bijou bottles were then aseptically filled with brain–heart infusion (BHI) broth, and the canals and access cavity infected with 30 μL of *Enterococcus faecalis* suspension made to a 0.5 McFarland standard in BHI broth. The access cavities were covered with a drop of mineral oil to prevent evaporation of inocula. Teeth and lids were again covered with tin foil and incubated at 37 °C for 48 h in 5% CO_2 atmosphere.

Protocol for test solution irrigation

Following incubation and biofilm growth, the teeth were treated with a measured amount (10 mL per canal) of test solution as irrigant whilst filing with a size 20 K Flex file (Kerr UK Ltd, Peterborough, UK) for 1 min. The canals were initially irrigated with the test solutions in a standard way using a 27 gauge needle reaching three quarter of the working length over 30 s and then the irrigant was slowly replaced by gentle syringing, the last volume of irrigant was left in place for 10 min with no agitation. In the ultrasonic groups, a size 20 file (EMS mini-piezon insert; Optident Ltd, Ilkley, UK) inserted to three quarter of the working length was used at lowest power setting to lightly brush the canal walls circumferentially at intermittent intervals after the initial irrigant replacement.

Measurement of antibacterial efficacy

At the end of the irrigation period, the canals were filled with 3 mL reduced transport medium (RTF) (Syed & Loesche 1972) to replace the test irrigant. The canals were then filed with a size 20 K Flex file for 20 s to working length. Three size 30 paper points were inserted into each canal and left for 1 min each to sample its contents. The paper points were removed to a 1.5 mL Eppendorf tube (Eppendorf-Nethelen-Hinz GmbH, Hamburg, Germany) containing 1 mL RTF giving the 'neat' sample which was serially diluted to 10^{-7} in 180 μL, transferring 20 μL each time; 50 μL were plated onto blood agar (plus 5% defibrinated horse blood), incubated at 37 °C for 24 h and enumerated to give residual colony-forming units (CFU) mL^{-1}. Baseline counts were established by sampling the biofilms in untreated teeth for the PBS group ($n = 24$).

Data and statistical analysis

The data were analysed by calculating ratios of residual CFUs in PBS versus the test irrigants (CFU counts from PBS/CFU counts from experimental group). These ratios were used as the dependent variables in a multivariate regression which was formulated as a multilevel model (Goldstein *et al.* 2002) using MLwiN (Rasbash *et al.* 2001). The ratios had to be logarithmically transformed to meet Normality assumptions. A 5% significance level was used.

Results

Descriptive analysis

The mean CFUs mL^{-1} for each group are presented in Fig. 1. It depicts the baseline control counts and the electrochemically activated solution groups with and without ultrasonication. The results show that all treatment groups had lower mean CFU counts than the mean baseline control counts. Sodium hypochlorite gave by far the lowest CFU counts, by a factor of 155 compared with PBS.

Statistical analysis

As the experimental groups were balanced by anatomical parameters, the absence of a single data point in one group as a result of random error meant that the remaining 'balancing' teeth had to be removed from the other groups. As a result, only a subset could be used for statistical analysis; of the potential 144 data points from eight test groups (X18), 20% were missing giving 115 valid data points. A multivariate approach was used to enhance statistical power and take account of any correlations between groups that may mask differences (McLeod 2001). The correlations between the dependent variables as estimated by the multivariate model are shown in Table 1.

Comparison of test irrigants with PBS control (Question 1)

All but two of the experimental groups were significantly different from their PBS controls (Table 2).

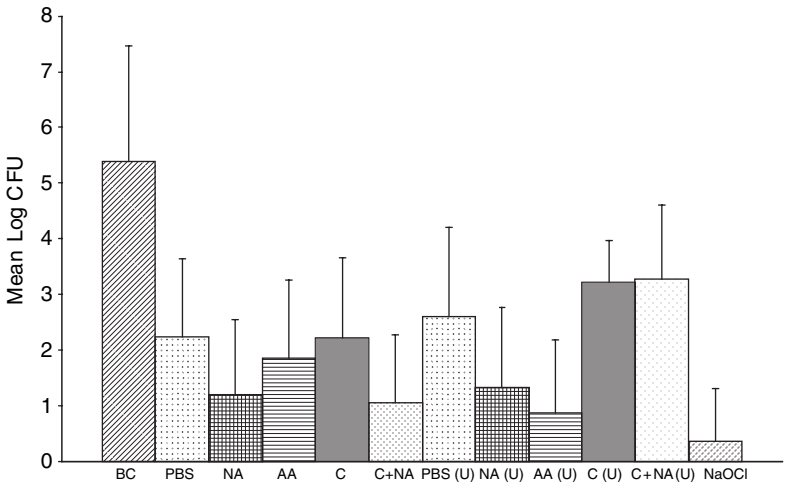

Figure 1 The mean CFUs mL^{-1} and standard deviations ($n = 18$ for all groups except BC, where $n = 24$) per experimental group are shown in this bar chart.

Table 1 The correlations between dependent variables as estimated by the multivariate model are shown for the eight experimental groups (−1 indicates perfect negative linear correlation and 1 indicates perfect positive correlation)

	No ultrasonication				Ultrasonication			
	Neutral anolyte	Acidic anolyte	Catholyte	Catholyte/ neutral anolyte	Neutral anolyte	Acidic anolyte	Catholyte	Catholyte/ neutral anolyte
No ultrasonication								
Neutral anolyte	1	0.65	0.35	0.47	0.57	0.48	0.74	0.65
Acidic anolyte		1	0.66	0.51	0.44	−0.14	0.42	0.60
Catholyte			1	0.22	0.34	−0.24	0.11	0.39
Catholyte/neutral anolyte				1	0.45	0.37	0.64	0.46
Ultrasonication								
Neutral anolyte					1	0.33	0.75	0.66
Acidic anolyte						1	0.73	0.32
Catholyte							1	0.65
Catholyte/neutral anolyte								1

Catholyte (ultrasonicated, U) and catholyte alternated with NA (U) both had *more* bacteria compared with PBS (U) controls (Fig. 1, Table 2). There were approximately 11× more bacteria in the PBS control (without ultrasonication) than the NA (without ultrasonication) group. Whilst, the NA (U) and acid anolyte (U) in comparison with the PBS (U) control consisted of approximately 13× and 70× less bacteria, respectively. The C/NA (with ultrasonication) had on average 12.5× more bacteria than the PBS (with ultrasonication). In contrast, the C/NA (without ultrasonication) had on average 17.5× fewer bacteria than PBS (without ultrasonication) (Fig. 1, Table 2).

Comparison of test irrigants with and without ultrasonication (Question 2)

There was a significant difference between the C alternated with NA groups with and without ultrasonication but not between other combinations (Table 3). PBS (with ultrasonication) resulted in almost 10× more bacteria than the PBS (without ultrasonication) (Table 3). There was a similar trend for greater numbers of bacteria in the ultrasonicated catholyte group compared with the nonultrasonicated group, although the difference was not significant.

Which was the most effective test irrigant? (Question 3)

To address this question, confidence intervals were constructed comparing each group with the group which was most effective in reducing the number of bacteria (Table 4). The nonultrasonicated groups were compared against the C alternated with NA group and the ultrasonicated groups were compared against the acid anolyte group. There were significant differences

Solution	Coefficient (95% confidence intervals)	Significance at $\alpha = 0.05$
No ultrasound PBS		
Neutral anolyte (NA)	10.91 (2.02–58.89)	S
Acid anolyte (AA)	1.82 (0.20–17.02)	NS
Catholyte (C)	0.70 (0.08–6.58)	NS
Catholyte/neutral anolyte (C/NA)	17.46 (2.19–139.44)	S
Ultrasound PBS (U)		
Neutral anolyte (NA)	13.46 (1.75–103.38)	S
Acid anolyte (AA)	70.11 (4.17–1178.97)	S
Catholyte (C)	0.12 (0.02–0.61)	S
Catholyte/neutral anolyte (C/NA)	0.08 (0.01–0.46)	S

Table 2 The results of the multivariate regression, framed to answer the first question: 'are the experimental solutions better than the control [phosphate buffered saline (PBS)]?' The estimated coefficients are given together with the 95% confidence intervals

Ultrasonicated versus nonultrasonicated	Coefficients (95% confidence intervals)	Significance at $\alpha = 0.05$
Neutral anolyte (NA)	0.81 (0.14–4.76)	NS
Acid anolyte (AA)	0.03 (<0.01–1.25)	NS
Catholyte (C)	6.11 (0.42–88.23)	NS
Catholyte/neutral anolyte (C/NA)	217.02 (26.31–1790.05)	S

Table 3 The results of the multivariate regression framed to answer the second question: 'are the experimental solutions activated with ultrasonication better than those used without ultrasonication?'

Table 4 The results of the multivariate regression framed to answer the third question: 'which is the best experimental solution (both with and without ultrasound)?' To address this question, confidence intervals were constructed comparing each group with the group which was most effective in reducing the number of bacteria

Groups compared	Coefficients (95% confidence intervals)	Significance at $\alpha = 0.05$
No ultrasonication		
Catholyte/neutral anolyte (C/NA) was most effective and acted as reference for comparison		
C/NA versus neutral anolyte	1.60 (0.21–12.30)	NS
C/NA versus acid anolyte	9.58 (1.03–89.12)	S
C/NA versus catholyte	24.78 (1.57–391.51)	S
Ultrasonication		
Acid anolyte (AA) was most effective and acted as reference for comparison		
AA versus neutral anolyte	5.21 (0.28–96.54)	NS
AA versus catholyte	601.85 (83.10–4359.01)	S
AA versus catholyte/neutral anolyte	862.64 (49.90–14 913.17)	S

between all but two comparisons (Table 4) and they involved either the C or C/NA groups.

What was the effect of tooth anatomy?

The effect of tooth anatomy measured by root length and tooth type was evaluated by assessing the effect of these as covariates in the multivariate regression model. The mean length for each group was fitted in the model as a covariate. The effect for all eight test groups was highly insignificant; this was either a true result or the analysis was underpowered. The same was true but to a lesser extent for tooth type. There were some significant differences in the acid anolyte (U) group and PBS (U) group, relating to premolar and lower incisor teeth but definitive conclusions could not be drawn.

Discussion

The antibacterial efficacy of the solutions was tested *in vitro* using a range of root canal isolates (Ghori *et al.* 2002). It is perhaps more relevant to test the effectiveness of the test solutions in a tooth model. *Enterococcus faecalis* was chosen as it is considered to be one of the most resistant species encountered in the root canal system (Molander *et al.* 1998, Sundqvist *et al.* 1998). It has also been shown to survive as a mono-infection in root canals (Moller *et al.* 1981, Fabricius *et al.* 1982) and is capable of eliciting a periapical host response

(Allard *et al.* 1987). The difficulty with the infected tooth model is that despite using standard inocula, variability is introduced by tooth anatomy and the individual growth responses within each tooth during the 48-h incubation period. As a result, the data showed considerable variation giving wide confidence intervals. The test model would require a considerably larger sample size to increase confidence in the estimates. Pilot studies had established that the 48-h time-frame was sufficient to develop single species biofilms, a more appropriate test model than one using broth cultured planktonic phenotypes. An attempt was made to account for the problem of variability of tooth anatomy matching the experimental groups for tooth length and estimated canal system 'volume'. In the final analysis some of the data were excluded because matching data points were missing. The anatomical factors were coded as covariates in the multivariate regression models. In each matched tooth group, there was a range of tooth lengths. For modelling purposes, the mean length was taken for each group and fitted in the multivariate regression model as a covariate. For all eight experimental groups, the effect of the tooth length covariate was highly insignificant. With this small data set it can only be stated that this indicates either truly no effect, or that there was not enough data to detect the effect.

To a lesser extent this is true when assessing tooth type, but some of the differences were large enough to be found statistically significant. The largest difference found was associated with the acid anolyte with ultrasonication group. Within this group, premolar teeth had almost 50% less bacteria than premolar teeth from the PBS with ultrasonication group. This may be compared with lower incisor teeth that had 1/44 757 times fewer bacteria than lower incisor teeth from the PBS with ultrasonication group. A similarly large difference was found between upper incisor and premolar teeth for the acid anolyte with ultrasonication group. Inspection of the raw data revealed that the sample size prohibited further inferences. Unfortunately, due to *relatively* small numbers the covariates could not be assessed with large statistical power. However, it can be tentatively concluded that there were some differences by tooth type. Although there were only small numbers of different tooth types, there is evidence that the differences in outcome between tooth types are not constant across all eight experimental groups.

As the canal shape had already been achieved (helping to standardize anatomy to an extent) before canal infection, the phase of debridement was not strictly true to 'real-life' and a compromise had to be adopted. A standard period of initial filing of the canal walls during irrigation was used, with the needle reaching a standard distance into the canal. The test solution was also allowed to soak into the canal anatomy for 10 min before its removal and replacement by RTF; it was hoped that this would simulate the period of effective action that may be achieved towards the end of canal preparation.

The properties of the anolyte solutions compared with catholyte solutions are quite different. Anolyte solutions (containing the following ionic species: Cl^2, HOCl, $ClO^{-\cdot}$ $ClO^{\cdot}$, $Cl^{\cdot}$, $HO_2^{\cdot}$, HO_2, O_2, $HO^{\cdot}$, O_3, $O_2^{\cdot}$, $^3O_2$,$^1O_2$, $O^{\cdot}$, H_3O^+, $H^{\cdot}$, H_2O_2, Cl^2O, ClO_2^-, HCl, Cl_2O^-, $S_2O_8^{8-}$, $C_2O_6^{2-}$, HClO, H_2SO_4, HSO_3Cl) possess an odour akin to bleach but are potable, whilst the catholyte solution [containing the following ionic species; OH^-, $H_3O_2^-$, O_2^-, HO_2^-, H_2O_2, H_2, HO, H_2^-, NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$] does not possess an obvious smell but has a 'soapy feel' (Marais 2000). It has been considered that the acid anolyte solution may be useful for removing debris and the smear layer whilst the catholyte solution may act as a detergent and is considered to be biocompatible (Ichikawa *et al.* 1999, Petrushanko & Lobyshev 2001, Serper *et al.* 2001).

The present study found a range of different effects on *E. faecalis* amongst the electrochemically activated solutions. Nonultrasonicated acid anolyte and catholyte had an effect no greater or lesser than that of PBS. In contrast, NA (with and without ultrasonication), C alternated with NA (without ultrasonication) and acid anolyte (with ultrasonication), all had significantly ($\alpha = 0.05$) lower CFUs compared with PBS controls. Whilst, C (with ultrasonication) and C alternated with NA (with ultrasonication) had significantly *more* CFUs than PBS controls (Table 2). The key to this observation appears to lie in the behaviour of the basic solutions against *E. faecalis* and the effect of ultrasound on their efficacy.

The anolyte solutions are essentially antibacterial towards *E. faecalis* although the neutral solution is more so. Upon ultrasonication, this effect is enhanced but interestingly is enhanced to a greater degree for the acid anolyte (Tables 2 and 4), although this difference was not found to be significant in these data (Table 4). It is hypothesized that the relative proportions of ionic species in the two anolyte solutions are different (as also indicated by the pH) but that ultrasonication enhances the bactericidal properties of some of these ionic species to a greater extent.

It is further hypothesized that catholyte has no enhanced ability to reduce CFUs in comparison with PBS; when ultrasonicated, both solutions presumably help to disrupt and disperse the bacterial biofilm resulting in the potential increase in CFUs. The difference between the solutions may lie in their relative abilities to sustain or kill the dispersed bacterial cells; those suspended in catholyte are presumably not as easily killed but somehow sustained, albeit passively. This would explain the greater numbers of bacterial cells in the ultrasonicated PBS (compared with nonultrasonicated) and the even greater numbers in the ultrasonicated catholyte.

It is difficult to compare the findings of this study with those that have evaluated the antimicrobial efficacy of electrochemically activated solutions (Horiba *et al.* 1999, Marais & Brozel 1999, Marais & Williams 2001, Prince *et al.* 2002) because of differences in methodology. Marais & Brozel (1999) and subsequently Walker *et al.* (2003) evaluated the ability of electrochemically activated water to control dental unit water line biofilms. Prince *et al.* (2002) successfully showed the ability of a commercially produced biocide (Aqualox® – electrochemically activated water [Aquabox System, Steribox Technologies Ltd, Abingdon, UK]) to control *Legionella* spp. and heterotrophic bacteria in industrial cooling towers. The ability of Sterilox® (electrochemically activated water [Sterilox Technologies, Sterilox Medical Ltd, Yardley, PA, USA]) to kill *Bacillus subtilis* spores has been demonstrated (Loshon *et al.* 2001).

Horiba *et al.* (1999) tested the antimicrobial efficacy of electrolysed water on 17 bacterial strains from root canal infections in an *in vitro* model. Marais & Williams (2001) used an infected tooth model (with an inoculum of four strains) to evaluate electrochemically activated water but did not allow incubation to develop biofilms. It can only be broadly stated that the solutions in these different studies under various circumstances showed antimicrobial potency.

This study has shown that electrochemically activated anolyte solutions (especially when ultrasonicated) have potential as root canal irrigants even if they are not as potent as 3% sodium hypochlorite. The potentially reduced toxicity and dentine weakening effects may prove to be advantageous. The most effective solution characteristics have yet to be found and these should also be tested for biocompatibility and effect on dentine.

Conclusions

Within the limitations of this study, it may be concluded that:

- The NA, NA (U), C alternated with NA and the AA (U) groups all had significantly ($\alpha = 0.05$) lower CFU counts compared with PBS controls.
- Ultrasonicated C and C activated with NA had significantly ($\alpha = 0.05$) higher CFU counts than PBS controls.
- Ultrasonicated C alternated with NA had significantly ($\alpha = 0.05$) higher CFU counts than its nonultrasonicated counterpart group.

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